



## Identification and characterization of thorny and high temperature tolerant brinjal (*Solanum melongena*) genotype for hot arid environment

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Received: 27 December 2023; Accepted: 30 September 2024

### ABSTRACT

Brinjal (*Solanum melongena* L.) is one of the most common vegetable which is generally cultivated as rainy (*kharif*) season crop to get the fruits during autumn-winter season. However, the summer season crop has more potential than the *kharif* season as the fruits harvested during summer gets premium price in the market. But, getting high quality fruit yield during summer due to high temperature stress (>45°C) is a challenge particularly in hot arid agro-climate as the day and night temperature above 35°C reduces the pollen viability, fruit set, number of fruits/plant and colour retention to a great extent. The present study was carried out during 2020 and 2021 at ICAR-Central Institute of Arid Horticulture, Bikaner, Rajasthan to develop varieties with better quality fruit yield under high temperature conditions. The experiments were conducted in split plot design (SPD) considering environments as main plots and replicates within environment as subplots with three replications. As a result, CIAH-22 has been identified and characterized as a trait specific high temperature tolerant (up to 45°C) thorny brinjal genotype suitable for hot arid region. Fruit weight at marketable stage varied from 144.56–155.84 g that indicates bigger sized fruits with thorns on calyx which are preferred most by the local growers and consumers of this region. It has the yield potential of 2.18–3.72 kg fruits/plant and 447.78–471.74 q/ha. The identified genotype CIAH-22 was able to set fruits at temperature as high as 45°C which was also evidenced by its pollen viability i.e. 83.71%. High pollen viability significantly enhanced fruit set, fruit weight and number of fruits per plant and thereby yield under high temperature stress. Thus, this identified genotype can be a potential source for brinjal improvement for high temperature tolerance and regional preference of big-sized thorny fruits with attractive colour retention.

**Keywords:** Arid, High temperature, *Solanum melongena*, Thorny fruits, Yield

Brinjal (*Solanum melongena* L.) is one of the most important solanaceous vegetable crop, which is a member of the Solanaceae family with chromosomal number 2n=2x=24 (Amin *et al.* 2014). Internationally, it is known as aubergine or eggplant. The primary point of origin and diversity for brinjal is thought to be India (Vavilov 1931, Bhaduri 1951). It is widely consumed by individuals from all social classes, earning the title "vegetable of the masses" (Patel and Sarnaik 2003 and Gogoi *et al.* 2018). In addition to being highly suggested as a treatment for diabetes patients and liver problems, aubergine is well known for its medicinal properties (Tiwari *et al.* 2009). The demand for eggplant has been rising quickly and steadily in recent years because of its many health advantages, including its antioxidant, anti-diabetic, hypotensive, cardioprotective, and hepatoprotective properties (Ojiewo *et al.* 2007).

The ideal temperature for eggplant growth and development is between 22 and 30°C. With global warming, temperatures in subtropical and tropical locations frequently exceed 35°C, resulting in major heat injuries in eggplant, namely reduced plant growth, lower yield, and poor quality (Dhatt and Kaur 2017). There are number of improved brinjal cultivars suitable for rainy season performing better due to favourable climatic conditions and low incidence of insect-pests (Nath *et al.* 2008). However, in summer season, brinjal has high demand due to premium price, but high temperature, low humidity, strong desiccating winds negatively affect fruit set (Singh and Kalda 2000). Brinjal fruit set is best at 18–21°C (Nath *et al.* 2008), while most cultivars experience reduced fruit set at temperatures above 35°C (Mohanty and Prusti 2000, Pandit *et al.* 2010 and Ansari *et al.* 2011). Expression of crucial reproductive characters under high temperature is responsible for heat tolerance, which is inherent to tolerant genotypes. Prolonged or even short duration exposure to high temperature affects normal plant functions. It affects all plant parts, including leaves, floral buds, and roots (Tsukaguchi *et al.* 2003). Brinjal which is the most widely grown vegetable in north-

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western India, is not yet fully exploited in terms of specific trait, quality, and region-specific variants (Samadia *et al.* 2019). The brinjal varieties that are presently in scattered cultivation are from favourable agro-climate of nearby states and recommended under the national programme in general, and these are not performing well in the hot arid areas of north-western part of Rajasthan due to extremeness of climatic conditions as well as susceptibility towards the high temperature and soil-moisture conditions of sandy soils. On the other hand, the local landraces or popular types with good fruit taste of consumer's preferences are very low yielding and susceptible to biotic and abiotic factors (Khan and Samadia 2016, Samadia and Haldhar 2019). Considering the above facts, the present study was aimed to identify the high temperature tolerant brinjal genotype with fruits having regional preference as well for developing trait specific cultivar for hot arid agro-climate.

#### MATERIALS AND METHODS

The present study was carried out during 2020 and 2021 at ICAR-Central Institute of Arid Horticulture, Bikaner ( $28^{\circ} 1' N$  latitude,  $73^{\circ} 18' E$  longitude at an altitude of 234.84 m above sea level), Rajasthan. The identified brinjal genotype CIAH-22 (IC-0635988) was evaluated under four different environments, viz. summer (February–June, 2020); *kharif* (July–November, 2020); summer (February–June, 2021); and *kharif* (July–November, 2021) for horticultural and yield traits. To evaluate the performance of a single variety across different environments, the experiments were conducted in split plot design (SPD) considering environments as main plots and replicates within environment as subplots

with three replications (Montgomery 2017). The spacing maintained between rows was 60 cm and between plants 40 cm. The experiments were raised on drip irrigation system in second fortnight of February for summer season and first fortnight of August for rainy season. Fertilization, irrigation, other cultural practices and need based plant protection measures were followed as recommended for commercial production. The mean weekly minimum and maximum temperature was recorded from sowing to last harvest of the crop (February–June and July–November) during the years 2020 and 2021 (Fig. 1). The observations were recorded on five randomly selected plants from each replication for plant growth, yield and yield contributing traits. Among plant growth parameters, the observations were recorded on plant height (cm) at 90 days after planting (DAP), no. of branches/plant, leaf length (cm), leaf width (cm), petiole length (cm), no. of thorns/leaf and no. of thorns on calyx/fruit. Among fruit yield and its components, no. of fruits/plant, fruit length (cm), fruit diameter (cm), fruit weight (g) at marketable stage (when fruits are immature and have glossy appearance), fruit yield/plant (kg) and fruit yield (q/ha) were assessed. The yield performance of CIAH-22 was also compared with the national checks, viz. Pusa Bindu, Pusa Kranti and Pusa Uttam over the environments. Statistical analysis of the data was done as per method prescribed by Montgomery (2017).

**Pollen viability studies:** The viability of the fresh pollen samples of brinjal genotype CIAH-22 was assessed during summer season using the acetocarmine technique, as detailed by Roberts (1977). Ten slides were used for each count of 100 pollen grains. Pollen grains with deep staining

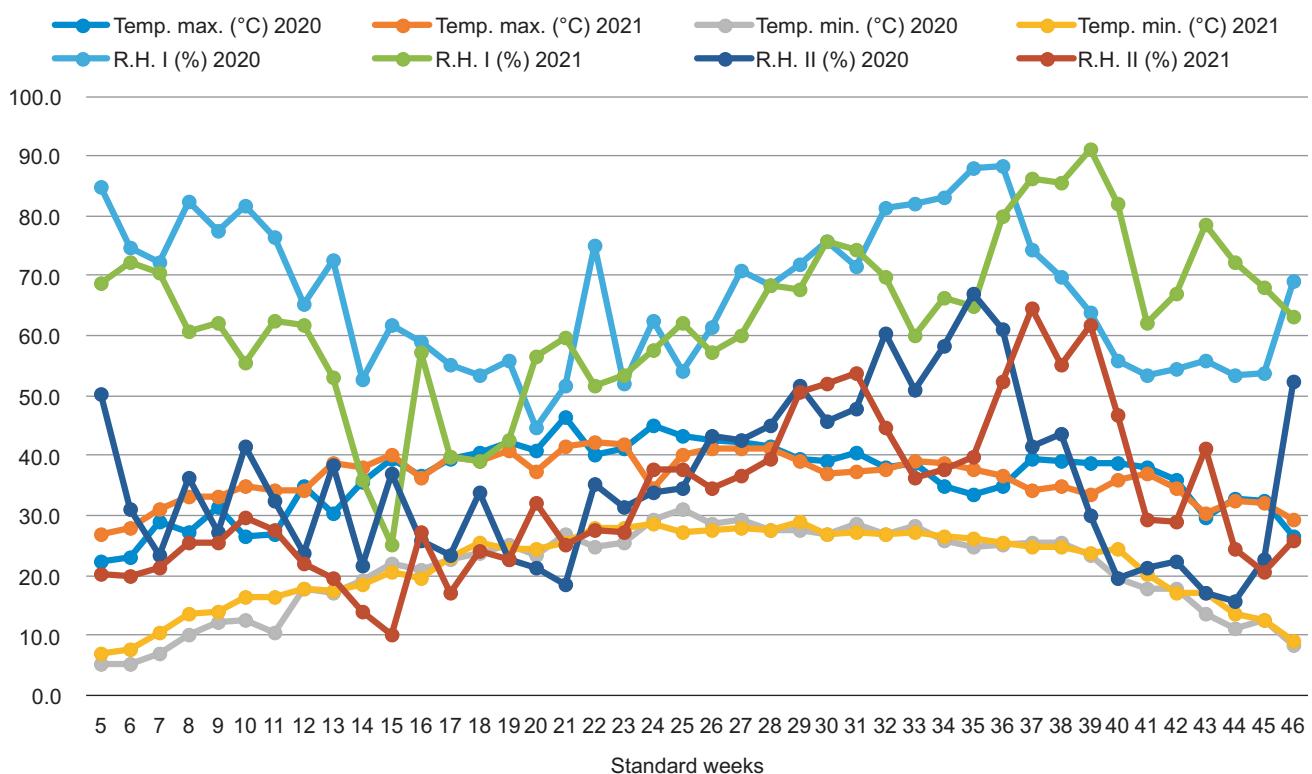


Fig. 1 Agro-meteorological parameters prevailing during the crop season (February–November, 2020 and 2021).

and normal appearance were deemed viable, whereas those with weak staining were tallied as non-viable (Pearson and Harney 1984). Based on this determination, the percentage of viable pollen (i.e. the number of stained pollens divided by the total number of pollens) was calculated.

**Molecular profiling:** Leaf samples from field-grown 12 brinjal genotypes including CIAH-22 were collected for their molecular characterization. The samples were then maintained at a deep freezer (-80°C) until DNA isolation. With a few small adjustments, 100 mg of leaf tissue was used to isolate DNA using the DNeasy Plant Mini Kit (Qiagen, Netherlands). DNA was evaluated electrophoretically on a 0.8% agarose gel and quantitatively using a spectrophotometer (GeneQuant, Pharmacia, France). Five CBDP and ten ScoT markers were chosen for custom synthesis by Eurofins Genomics, India Pvt. Ltd., Bangalore, from the investigations of Collard and Mackill (2009) and Singh *et al.* (2014), respectively. The CBDP and ScoT markers were profiled through PCR reaction using a master mix comprising 2.0  $\mu$ l of 10X Taq DNA polymerase buffer, 0.5  $\mu$ l of dNTP mix (10 mM), 1.0  $\mu$ l of MgCl<sub>2</sub> (25 mM), 2.0  $\mu$ l of primer (10 mM), 1U of Taq DNA polymerase enzyme (Genetix Biotech Asia Pvt. Ltd., India), 3.0  $\mu$ l of template DNA (20 ng/ $\mu$ l), and nuclease-free water (Hi-media, India) as the final volume was adjusted to 20  $\mu$ l. Singh *et al.* (2014)'s touch-down PCR programme was used for the PCR amplification of CBDP markers. For the ScoT markers, the thermal profile was as follows: a 3 min initial denaturation at 94°C was followed by 40 cycles of denaturation at 94°C for 30 sec, primer annealing at 45°C for 30 sec, and extension at 72°C for 2 min. For ten minutes, the final extension for both kinds of markers were carried out at 72°C, and then it was held at 10°C. Using a 1 kb standard DNA ladder, the PCR amplicons of the CBDP and ScoT markers were electrophoretically examined on a 1.2–1.5% agarose gel (Fermentas USA). In order to identify the CIAH-22 genotype from the remaining comparative controls, the particular band associated with this genotype was highlighted as DNA fingerprints.

## RESULTS AND DISCUSSION

During both the years, wide variations were experienced in all the weather parameters. The weekly minimum and maximum temperatures during summer season (February–June) ranged between 5.2°C (5<sup>th</sup> standard week) and 44.9°C (24<sup>th</sup> standard week), respectively, in the year 2020. While, during *kharif* season (July–November), these ranged between 11.3°C (44<sup>th</sup> standard week) and 40.5°C (31<sup>st</sup> standard week). Similarly, during the year 2021, the weekly minimum and maximum temperatures during summer season (February–June) ranged between 7.1°C (5<sup>th</sup> standard week) and 41.1°C (26, 27 and 28<sup>th</sup> standard week), respectively. While, during *kharif* season, these ranged between 13.6°C (44<sup>th</sup> standard week) and 39.1°C (33<sup>rd</sup> standard week). The coolest temperatures during both the years were observed in the months of February while the month of June recorded the highest temperatures. From the weather data, it is clearly

evident that the high temperature range was 39.1–44.9 over the environments and years which is much higher than the optimum temperature range (21–30°C) required for normal growth and development of plant and fruits in brinjal. Even under such harsh environment, the genotype CIAH-22 (IC-0635988) was found high temperature (up to 45°C) tolerant as there was continuous fruiting during hot summer months (April–June) and high yield with quality fruits (size and colour retention) was obtained (Anonymous 2020a, Anonymous 2020b, Anonymous 2021).

Important horticultural and yield related traits of the brinjal genotype CIAH-22 are depicted in Table 1, which shows its overall performance under hot-arid region. The four seasons evaluation results showed that CIAH-22 exhibited plant height ranged from 43.06–60.10 cm with number of branches from 3.0–4.9. The leaf length (cm), leaf width (cm) and petiole length (cm) were varied from 18.83–21.63, 14.93–15.94 and 5.35–6.13, respectively (Table 2). Presence of thorns is the unique trait of this genotype and number of thorns on calyx/fruit (12.28–20.00) was found higher than that of leaves (4.70–8.00). For yield and yield contributing traits, number of fruits per plant was varied from 16.82–27.58 (Table 3). Observations regarding fruit length and diameter at marketable stage varied from 7.56–8.57 and 6.94–7.44, respectively. Results pertaining to fruit weight at marketable stage varied from 144.56–155.84 g that indicates bigger sized fruits with thorns on calyx. CIAH 22 gave a fruit yield of 458.5–471.7 q/ha over the environments and years with non-significant differences which clearly showed that the genotype is stable yielder. Yield is a major selection criterion for selecting the desirable varieties/genotypes under high temperature stress and therefore, the performance of the identified genotype was also compared with the national checks, viz. Pusa Bindu, Pusa Kranti and Pusa Uttam which recorded the yield range of 301.6–348.2, 284.4–328.1 and 289.8–312.8 q/ha, respectively. Thus, it was found that CIAH-22 recorded 52.02–35.47, 61.22–43.77 and 58.21–50.80% higher yield

Table 1 Salient characteristics/chief botanical and horticultural description of brinjal genotype CIAH-22

Trait	Description
Plant height at 90 DAP (cm)	43.06–60.10
No. of branches/plant	3.0–4.9
Leaf length (cm)	18.83–21.63
Leaf width (cm)	14.93–15.94
Petiole length (cm)	5.35–6.13
No. of thorns/leaf	4.70–8.00
No. of thorns on calyx/fruit	12.28–20.00
No. of fruits/plant	16.82–27.58
Fruit length at marketable stage (cm)	7.56–8.57
Fruit diameter at marketable stage (cm)	6.94–7.44
Fruit weight at marketable stage (g)	144.56–155.84
Fruit yield/plant (kg)	2.18–3.72

DAP, Days after planting.

Table 2 Plant growth parameters of brinjal genotype CIAH-22 over the environments

Season/year \ Trait	Plant height at 90 DAP (cm)	No. of branches/plant	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	No. of thorns /leaf	No. of thorns on calyx/fruit
Kharif, 2020	57.45	4.16	21.63	15.38	6.13	7.13	18.77
Summer, 2020	43.06	3.00	18.83	14.78	5.73	4.70	13.44
Kharif, 2021	60.10	4.90	21.07	15.94	6.01	8.00	20.00
Summer, 2021	47.91	3.38	18.70	14.93	5.35	5.07	12.28
Average	52.13	3.86	20.06	15.26	5.81	6.22	16.12
CD (0.05)	6.41	1.56	3.22	2.29	1.90	2.31	2.20
CV (%)	5.38	1.50	5.94	4.72	1.38	1.87	2.24

Table 3 Fruit yield parameters of brinjal genotype CIAH-22 over the environments

Season/year \ Trait	No. of fruits/plant	Fruit length at marketable stage (cm)	Fruit diameter at marketable stage (cm)	Fruit weight at marketable stage (g)	Fruit yield/ plant (kg)	Fruit yield (q/ha)
Kharif, 2020	24.14	8.57	7.31	147.58	3.42	467.14
Summer, 2020	16.82	7.56	6.89	144.56	2.18	447.78
Kharif, 2021	27.58	8.12	7.44	155.84	3.72	471.74
Summer, 2021	18.15	7.36	6.94	140.71	2.36	458.52
Average	21.67	7.90	7.14	147.17	2.92	461.30
CD (0.05)	2.40	2.13	1.82	2.24	0.33	N/A
CV (%)	2.55	1.72	1.42	8.15	1.45	7.76

range than the checks, viz. Pusa Bindu, Pusa Kranti and Pusa Uttam, respectively. The difference in fruit yield between CIAH-22 and national checks was due to high and low fruit set, respectively under high temperature conditions of arid region. Similar reductions (even up to 90 %) in fruit yield of brinjal genotypes susceptible to high temperature as compared to tolerant genotypes have also been reported by Santhiya *et al.* (2019).

The average weight of fruits, fruit length, fruit diameter and no. of fruits/plant have also shown some extent of decrease in the summer season (140.71–144.56 g, 7.36–7.56 cm, 6.89–6.94 cm and 16.82–18.15, respectively) under high temperature stress conditions, compared to rainy season (147.58–155.84 g, 8.12–8.57 cm, 7.31–7.44 cm and 24.14–27.58, respectively) (Table 3). Similar results were reported by Mohanty and Prusti (2000) and Akhtar *et al.* (2017). Drastic lowering in fruit yield under high temperature owing to low fruit-set was reported by several workers (Kumar *et al.* 2000, Mohanty and Prusti 2000, Akhtar *et al.* 2017, Dhatt and Kaur 2017, Samadia *et al.* 2019, Santhiya *et al.* 2019), while CIAH-22 was capable to produce good yield (more than 2 kg fruits/plant) even in summer season when temperature goes above 45°C.

Observing normal reproductive behaviour under high temperatures, pollen viability study was conducted in these genotypes in the 44<sup>th</sup> standard week of the year 2020 when the maximum temperature was 45°C. Genotype CIAH-22 had normal pollen behaviour (Fig. 2). It had 83.71% pollen viability at 45°C temperature. The percentage of fruit set and fruit yield of CIAH-22 was also significantly higher than the other genotypes. These results further supported

the high temperature tolerance of this genotype as there was more than 80% pollen viability. Therefore, it is evident that the brinjal genotype CIAH-22 has tolerance to high temperature as suggested by profuse growth, good pollen viability and good fruit set even at extreme high temperature. Pollen viability is often used as a measure of a plant's thermotolerance and cultivars with high pollen viability under heat stress conditions produce more fruit in the field as reported by Miller *et al.* (2021) in tomato and Verma *et al.* (2023) in cowpea. The positive effect of high pollen viability on high fruit set, more fruits per plant and fruit yield and vice-versa has also been reported by Dhatt and Kaur (2017) in brinjal.

Molecular marker based characterization of a genotype becomes important to correctly identify the particular genotype from related varieties/genotype. To obtain the specific alleles in brinjal CIAH-22, ten Start Codon Targeted Polymorphism (ScoT) and 5 CAAT Box-derived Polymorphism (CBDP) markers were profiled on genomic DNA of 12 brinjal genotypes including thorny brinjal

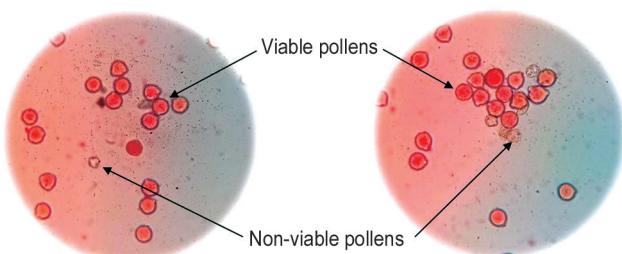


Fig. 2 Pollen viability studies in brinjal genotype CIAH-22.

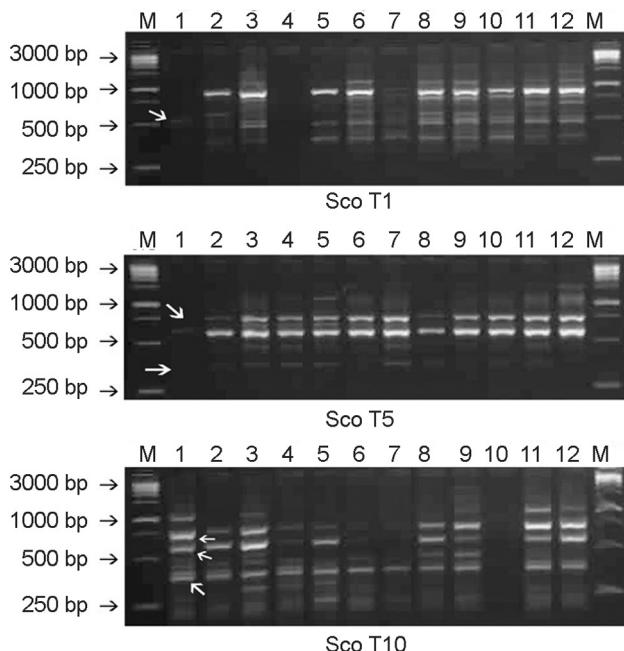


Fig. 3 Molecular profiling of brinjal genotype CIAH-22 with ScoT markers (No. 1 represents CIAH-22).

(Fig. 3). The alleles common to all 12 analyzed brinjal genotypes were considered monomorphic and hence not used as informative and specific allele to the brinjal CIAH-22. On the other hand, the allele(s) which was amplified at variable position and only present in CIAH-22 was considered as specific allele. Hence, the specific allele to brinjal CIAH-22 were good enough to distinguish and identify this genotype of the brinjal to rest of the analyzed control samples. In this investigation, three ScoT markers namely ScoT1, ScoT5 and ScoT10 amplified one to four specific alleles to the brinjal genotype CIAH-22. The ScoT1 and ScoT5 has produced at least one specific allele (approx. 610 and 550 bp, respectively), whereas ScoT10 has produced 4 specific alleles (approx. 350, 400, 550 and 700 bp). The CBDP markers did not produce any specific alleles in the thorny genotype of the brinjal. Thus, the CIAH-22 genotype of brinjal is different from the comparative control samples based on these specific alleles. A similar study was done by Sultana *et al.* (2018) in eleven brinjal genotypes with RAPD markers. Biswas *et al.* (2009) also observed molecular diversity among 10 promising brinjal varieties with RAPD markers.

Based on the crop phenology, yield traits and pollen viability studies over the season and years, a trait specific brinjal genotype CIAH-22 was identified as high temperature tolerant to produce quality yield under hot arid region. It also showed the traits as bigger fruit size with attractive bluish-purple colour, thorns on stem, leaves as well as fruit calyx. The identified genetic stock has potential for cultivation during rainy-winter and spring-summer season under abiotic stressed environment. Thus, the identified genotype CIAH-22 could be used in a breeding programme of brinjal to develop high temperature tolerant cultivars in

a changing climatic scenario.

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